

**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (Currently Amended) A method for constitutive and/or inducible gene knock down in a non-human vertebrate, ~~or in a tissue culture or cells of a cell culture derived from a non-human vertebrate~~, which comprises stably integrating by homologous recombination an expression vector into a polymerase II dependent locus of the genome of the non-human vertebrate, said expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter ~~into~~ and homologous sequences which integrate through homologous recombination at a polymerase II dependent locus of the genome of the non-human vertebrate, ~~of the tissue culture or of the cells of the cell culture~~ wherein the ubiquitous promoter is selected from the group consisting of polymerase I, II and III dependent promoters.

2. (Canceled)

3. (Canceled)

4. (Canceled)

5. (Previously Presented) The method of claim 1, wherein the polymerase II dependent locus is selected from the group consisting of a Rosa26, collagen, RNA polymerase, actin and HPRT locus.

6. (Previously Presented) The method of claim 1, wherein the expression vector further contains functional sequences selected from the group consisting of splice acceptor sequences, polyadenylation sites, and selectable marker sequences.
7. (Canceled)
8. (Currently Amended) The method of claim 7 1, wherein the ubiquitous promoter is a polymerase II or III dependent promoter.
9. (Currently Amended) The method of claim 7 1, wherein the ubiquitous promoter is selected from the group consisting of a CMV promoter, a CAGGS promoter, a snRNA promoter ~~such as U6~~, a RNase P RNA promoter ~~such as H1~~, a tRNA promoter, a 7SL RNA promoter, and a 5 S rRNA promoter.
10. (Original) The method of claim 1, wherein the ubiquitous promoter is a constitutive promoter.
11. (Original) The method of claim 1, wherein the ubiquitous promoter is an inducible promoter.
12. (Original) The method of claim 11, wherein the inducible promoter is a promoter containing a operator sequence selected from the group consisting of tet, Gal4, and lac.
13. (Previously Presented) The method of claim 1, wherein said non-human vertebrate is a mouse or fish.

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14. (Previously Presented) The method of claim 13, wherein said non-human vertebrate is a mouse.

15. (Previously Presented) The method of claim 1, wherein the expression vector is a Pol III dependent promoter driven shRNA construct to be integrated into a ubiquitously active Pol III dependent locus.

16. (Original) The method of claim 15, wherein the promoter is a constitutive H1 or U6 promoter.

17. (Original) The method of claim 15, wherein the promoter is an inducible U6 or H1 promoter.

18. (Previously Presented) The method of claim 1, wherein the expression vector is a Pol II dependent promoter driven shRNA construct to be integrated into a ubiquitously active Pol II dependent locus.

19. (Original) The method of claim 18, wherein the promoter is an inducible CMV promoter.

20. (Original) The method of claim 1, wherein the shRNA comprises at least one DNA segment

A-B-C

wherein

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A is a 15 to 35 bp DNA sequence with at least 95% complementarity to the gene to be knocked down;

B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hair pin molecule, and

C is a 15 to 35 bp DNA sequence with at least 85% complementarity to the sequence A.

21. (Original) The method of claim 20, wherein A is a 19 to 29 bp DNA sequence.

22. (Original) The method of claim 20, wherein the DNA sequence A has 100% complementarity to the gene to be knocked down.

23. (Original) The method of claim 20, wherein C is a 19 to 29 bp DNA sequence.

24. (Currently Amended) The method of claim 1, wherein the shRNA comprises a stop and/or polyadenylation sequence.

25. (Canceled)

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26. (Previously Presented) The method of claim 1, wherein the method for constitutive and/or inducible gene knock down in a non-human vertebrate comprises integrating the expression vector into ES cells of the non-human vertebrate.

27. (Currently Amended) A non-human vertebrate, ~~or tissue or cell culture derived from a non-human vertebrate~~ having stably integrated by homologous recombination at a polymerase II dependent locus of the non-human vertebrate, ~~tissue culture or cells of the cell culture~~, an expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter and homologous sequences which integrate at a polymerase II dependent locus of the genome of the non-human vertebrate, wherein the ubiquitous promoter is selected from the group consisting of polymerase I, II and III dependent promoters.

28. (Canceled)

29. (Currently Amended) The non-human vertebrate of claim 27, which is ~~or is derived from~~ a mouse or fish.

30. (Previously Presented) An expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter and homologous sequences which integrate at a polymerase II dependent locus of the genome of a non-human vertebrate, wherein the ubiquitous promoter is selected from the group consisting of polymerase I, II and III dependent promoters.

**CONDITIONAL PETITION FOR EXTENSION OF TIME**

If entry and consideration of the amendments above requires an extension of time, Applicants respectfully request that this be considered a petition therefor. The Commissioner is authorized to charge any fee(s) due in this connection to Deposit Account No. 14-1263.

**ADDITIONAL FEE**

Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

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